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Limbic Frontal Cortex in Hominoids: A Comparative Study of Area 13

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ABSTRACT The limbic frontal cortex forms part of the neural substrate responsible for emotional reactions to social stimuli. Area 13 is one of the cortical areas long known to be part of the posterior orbitofrontal cortex in several monkey species, such as the macaque. Its presence nevertheless in the human brain has been unclear, and the cortex of the frontal lobe of the great and lesser apes remains largely unknown.

In this study area 13 was identified in human, chimpanzee, bonobo, gorilla, orangutan, and gibbon brains, and cortical maps were generated on the basis of its cytoarchitecture. Imaging techniques were used to characterize and quantify the microstructural organization of the area, and stereological tools were applied for estimates of the volume of area 13 in all species.

Area 13 is conservative in its structure, and features such as size of cortical layers, density of neurons, and space available for connections are similar across hominoids with only subtle differences present. In contrast to the homogeneity found in its organization, variation is present in the relative size of this cortical area (as a percentage of total brain volume). The human and the bonobo include a complex orbitofrontal cortex and a relatively smaller area 13. On the contrary the orangutan stands out by having a shorter orbitofrontal region and a more expanded area 13.

Differences in the organization and size of individual cortical areas involved in emotional reactions and social behavior can be related to behavioral specializations of each hominoid and to the evolution of emotions in hominids. Am J Phys Anthropol 106:129–155, 1998.

The orbitofrontal cortex of primates has been an odd bedfellow to the motor and association cortices of the frontal lobe. Clearly, it is neither a motor (or motor-related) cortex nor a classical association cortex, having few "higher-order" functional attributes ascribed to it (Eslinger and Damasio, 1985). Set very much apart from the dorsolateral prefrontal cortex, the orbitofrontal cortex has been linked more closely to what could be termed "limbic" or "emotional" mechanisms (Damasio et al., 1990; Kling and Steklis, 1976). Stimulation or destruction of the posterior orbitofrontal area is associated with alterations in autonomic,

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emotional, and social behaviors (Damasio and Van Hoesen, 1983). Thus, area 13 along with the surrounding posterior orbitofrontal cortex constitutes a significant part of the neural substrate responsible for behaviors related to responses to social stimuli and social cognition.

On neuroanatomical grounds some parts of the orbitofrontal cortex deserve designation as part of the limbic lobe, including proisocortical areas (areas that lack a granular layer typical of prefrontal association cortices). Nevertheless, the largest expanse of the orbitofrontal cortex seems very much "prefrontal," sharing substantial granularity and a network of cortical connections which link it with frontal, parietal, and temporal association neural systems (Van Hoesen, 1982; Goldman-Rakic, 1984). Posterior orbital areas, by contrast, are strongly interconnected to the insular, temporal polar, and parahippocampal cortices as well as with basal forebrain structures like the ventral striatum, nucleus basalis of Meynert, and amygdala (Nauta, 1962; Van Hoesen, 1981).

Area 13 forms the core posterior orbitofrontal area, and its cytoarchitecture and connections are now well described in the macaque monkey (Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Morecraft et al., 1992; Carmichael and Price, 1994). Less is known about it in the human brain, and virtually nothing is known about it in the brains of the apes.

Here we compare area 13 using conventional and quantitative cytoarchitectonic techniques in all extant hominoids and one monkey species. In particular:

1. Cortical maps of area 13 are generated for human, chimpanzee, bonobo, gorilla, orangutan, and gibbon brains. A qualitative microscopic analysis is presented from serial sections stained with the Gallyas stain for cell bodies (cytoarchitecture), including a photographic documentation of the area in the above species.

2. The size of area 13 in hominoids is estimated using stereological tools for an unbiased estimate of its volume.

3. The microstructural organization of area 13 is investigated using quantitative cytoarchitectonic techniques. These include a) the application of a quantitative imaging technique (the Grey-Level Index method or GLI), which yields the ratio between neuropil space and space occupied by cellular somata, for the whole cortex and for individual cortical layers; b) the relative width of the cortical layers; c) the total number of neurons and their density.

Changes in emotional states and disinhibition of emotional reactions have been associated with area 13 in the macaque. Is area 13 present in all hominoids? Are there species-specific structural features that can be identified in the neural circuitry underlying complex social behaviors in apes and humans? Are there differences in size of this cortical area among species with different social organizations?

MATERIALS AND METHODS

Materials

All extant hominoids (humans, chimpanzees, bonobos, gorillas, orangutans, and gibbons) were studied, as well as one Old World monkey, the rhesus monkey. Two to ten hemispheres per species were included in the qualitative part of the investigation, and sections from one hemisphere (right hemisphere in all species) were digitized and quantified for each hominoid species.[1]

Histological sections from all the collections (see footnote 1) were used in the qualitative investigation, whereas the quantitative techniques were applied only to the new specimens (described under e) that were all embedded in paraffin, serially sectioned at 20 μm, and every tenth or 15th section was stained with a modification of the Gallyas silver stain for neuronal perikarya (Gallyas,

[1]The material investigated forms part of the following collections: a) two human brains and two macaque brains are part of G.H. Van Hoesen's collection; b) brains from one chimpanzee, one gorilla, one orangutan, and one gibbon are part of E. Armstrong's collection at the Armed Forces Institute of Pathology (A.F.I.P); c) one human brain, two macaque brains, and one chimpanzee brain are part of the Yakovlev Collection at the A.F.I.P. (all brains are sectioned at 20 or 35 μm, stained either with Nissl, for neuronal perikarya, or silver stain); d) three human brains, one macaque brain, one chimpanzee, one gorilla, and one hemisphere of the orangutan are part of K. Zilles' and H. Stephan's primate brain collection at the Vogt Institute for Brain Research in Duesseldorf, Germany; and e) five new ape brain specimens from the Yerkes Primate Regional Research Center and the Bush Gardens Zoo form part of K. Semendeferi's primate brain collection at UCSD (chimpanzee Chimp1, bonobo YN 86-137, gorilla YN 82-140, orangutan YN85-38, and gibbon YN 81-146). The specimens belong to adult individuals of both sexes, except for the bonobo (a 2-year-old female).

1971; Merker, 1983). The human brain included in the quantification (male, 75 years old) was processed like the ape specimens. None of the monkeys, apes, or humans died of neurological disease.

Methods

Estimates of volumes. After area 13 was identified (see Results) and outlined, its volume and that of the total volume of the brain were estimated on the histological sections. Stereological techniques which estimate unbiased volume of brain structures and other irregularly shaped objects, such as the cortex, were used (Gundersen et al., 1988a,b).

A grid test system of regularly spaced points on an acetate sheet was superimposed randomly on 10 to 15 coronal sections of known intersection distance, and of which the first one was randomly determined. On these sections, all the points P(struct) that fell within the hemispheres and cerebellum as well as within the boundaries of area 13 were counted. The absolute area of the cross section of each region of interest was determined by

$$A(s) = [P(struct)/P(total)]\,[A(p)/M^2]$$

where A(s) is the area of the region of interest, P(struct) is the number of points counted from that region, P(total) is the total number of grid points, A(p) is the total grid area, and M is the magnification of the section.

The total volume of the brain and of area 13 was estimated by applying the following:

$$V = A \times T \times D$$

where V is the volume of the region, A is the area of the cross section, T is the thickness of each section, and D is the distance between the sections. The above procedure provided an unbiased estimate of the volume of the brain and of cortical area 13 of the human and ape brains, with a precision better than 5% (Gundersen et al., 1988a,b).

Comparison of the brain volume prior to processing and the volume of the brain tissue as estimated from the above method produced a correction factor for each brain. The correction factor was applied to the volume of area 13. This factor corrects for the effects of shrinkage during processing of the tissue, as discussed by Stephan (1961), Stephan et al. (1991), and Frahm (personal communication).

Grey-Level Index (GLI). The Grey-Level Index (GLI) method (Schleicher and Zilles, 1989) detects the area-percent of stained perikarya versus neuropil, and demonstrates interareal and interlaminar differences in these densities across species. The GLI values include neuronal cell bodies, glial nuclei, and endothelial cell nuclei. The regional and laminar changes in cortical GLI are primarily a function of neuronal volume density, because glial and endothelial cell nuclei constitute a relatively small portion of the total volume, and because the packing density of glial cells does not differ significantly between the cortical layers (Wree et al., 1982; Armstrong et al., 1986). The GLI is defined by the following formula:

$$GLI = [A/F] \times 100$$

where GLI is the grey-level index in percent, A is the absolute area of the cellular profiles in the measuring field, and F is the area of the measuring field (here 20 × 20 μm). Determining GLIs requires three major steps (Fig. 1):

I. Histological sections were digitized, and images were obtained with a CCD-Camera attached to a light microscope. The GLIs were measured automatically with an IBAS 2000 image analyzer. The darkly stained cellular profiles were segmented from the background by adaptive thresholding, a procedure based on the relative grey value difference between the background and the cellular profiles, and independent of staining intensity. The cellular profiles include the cytoplasm, nucleus, and nucleolus of both neurons and glia (Fig. 1A–C).

A total of 12 locations in cortical area 13 of the seven quantified hemispheres were selected. The selection of sites was based mainly on the quality of the histological section and the orientation of the layers (non-tangential cuts). Selected locations, with a maximum size of 9 mm × 9 mm, were marked and outlined on the slides.

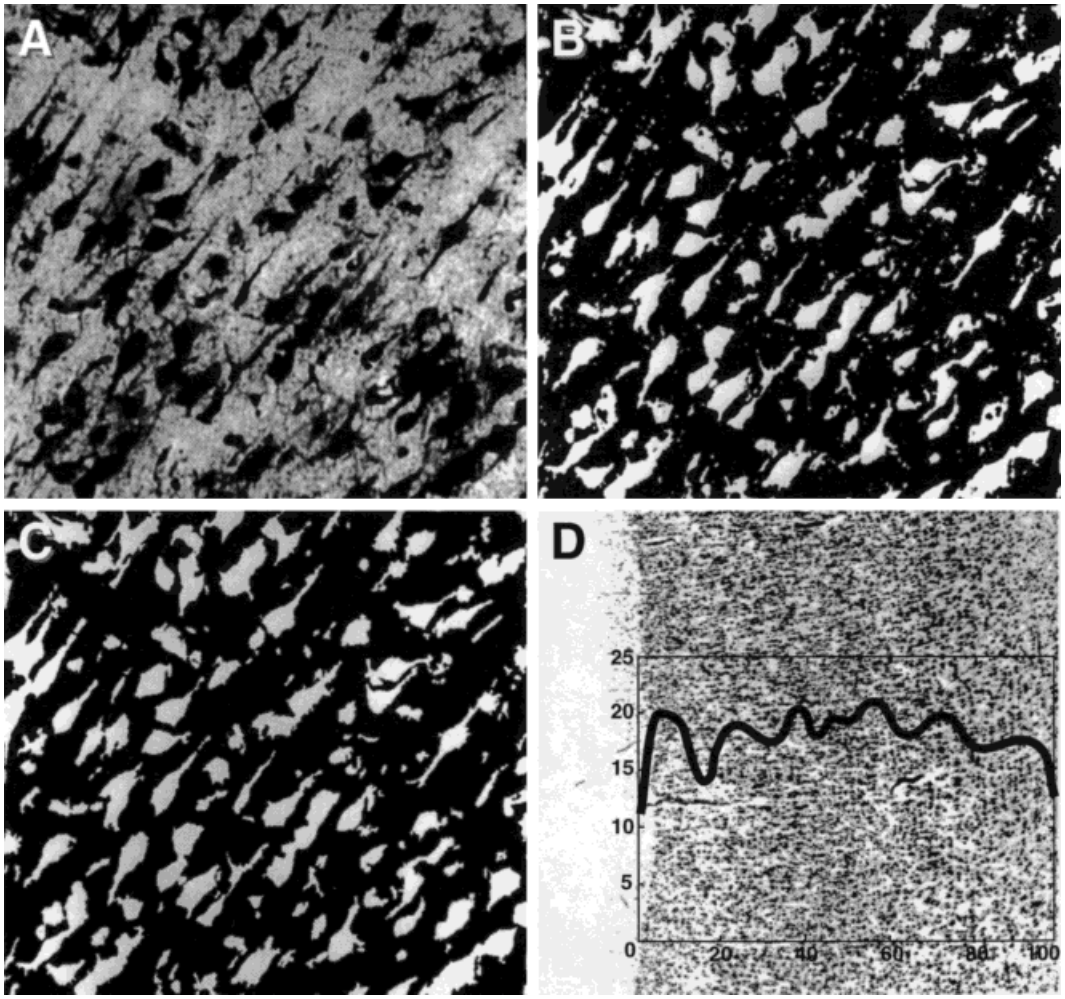

Fig. 1. Grey-Level Index method. **A:** Histological section in the light microscope. **B,C:** Histological section digitized. The darkly stained cellular profiles are segmented from the background. **D:** Profiles are drawn from the pial surface to white matter.

The microscopic image (25×) was transmitted through the CCD-Camera to the image analyzer (Fig. 1D). The video frame of 512 × 512 pixels covered a square microscopic field of 165 μm^2 wide. Each video frame included a set of 8 × 8 square measuring fields, and each measuring field was 20 × 20 µm (spatial resolution of 20 µm). After all the fields in each video frame were measured, the histological section was moved by a mechanical scanning stage to the immediately adjoining non-overlapping video frame. A maximum of 10 hr was needed to measure a large rectangle on the histological section. The data were stored in a picture matrix and printed. The resulting computer plots from the seven primate species show the regional distribution of the GLIs with a spatial resolution defined by the size of a measuring field (20 × 20 µm).

II. The above computer images and plots were used to generate laminar profiles of the cell distribution. The profiles describe variation in cell body volume density (GLI) throughout the cortical depth, starting at the pial surface and ending in the white matter.

Two straight lines at a distance of 23 to 50 pixels from each other and perpendicular to the pial surface, were drawn on each com-

puter plot, from the pial surface to the white matter. Then, in order to superimpose the lines on the picture matrix, each plot was fixed to a digitizing tablet, and the corresponding digitized image was presented in the monitor. Three corner points of the plot were marked, so that the digitized coordinates could be transformed to pixel coordinates. The lines were traced over with the mouse, and the GLI values were stored. The entire cortical segment included between the two lines was averaged, resulting in one thick profile for each selected location (Fig. 1D).

III. A characteristic profile for each species was derived by standardizing individual thick profiles to the same relative width, then averaged. The x-axis represents the relative cortical depth, and the y-axis represents the GLI value which shows the area percentage covered by cell bodies versus neuropil space.

Subsequently, mean GLI values were calculated for the entire profile, the supragranular layers (II and III), layer IV, and the infragranular layers (V and VI).

Relative size of cortical layers. The above profiles helped to determine the thickness of the cortical layers and the mean GLI values for each layer. The cortex in the microscopic slides was projected through a camera lucida over each profile, and the borders of layers were outlined for area 13 in all seven species. This allows a direct superimposition of the profile curves onto the image of the histological section. Thereby, the maxima and minima of the profile can be correlated with the cytoarchitectonically defined layers in each area across species.

Neuronal counts. The total number of neurons was determined for area 13 by applying the optical fractionator, a modern stereological method for obtaining estimates of total numbers of neurons in a cortical area (West and Gundersen, 1990; West et al., 1991) (Hyman, personal communication). Neurons were identified and counted in a light microscope using a 60× oil immersion objective with a numerical aperture of 1.4. The microscopic image was transmitted through a CCD-Video camera to a computer screen at a total magnification of 2,056×. Superimposed on the screen was a transparency of a counting square with extended exclusion edges drawn on it.

Neurons were separated from glial cells because the former are larger in most cases, and their cell nuclei are surrounded by stained cytoplasm, which is hardly visible in glial cells. Moreover, with the Gallyas cell stain, one can identify the initial segments of proximal dendritic processes in most neurons. The close association of some glial cells with capillaries is easily seen, because the latter are stained and are very distinct. Nevertheless, there were occasional instances where it was unclear whether the structure represented a neuron, and so throughout this research only those structures were counted that were clearly identified as neurons.

First, density of neurons was determined by counting neuronal bodies in a known volume of tissue. The standard volume of tissue in this case had a 100 micron X-axis, 100 micron Y-axis (drawn on the transparency superimposed on the screen), and 20 microns Z-axis that equals the thickness of the tissue sections. All neurons included in the box were counted, except those touching the three exclusion planes (X and Y axes on the screen) and the first level of focus (on the Z axis).

Second, a number of disectors was chosen according to a systematic random scheme (Hyman, personal communication) across the cortical area under investigation. A total of 50 disectors or more per cortical area per species were included in the measurements. This figure involved either every or every other stained section in the series, depending on the extent of the cortical area in the particular species. As a first step, the cortical area of each species was measured by the x and y coordinates attached to the microscope. The sampling of the boxes was accomplished with the help of a software developed for these measurements. The square area of the cortex under consideration was given to the computer as x and y axes, as well as the number of boxes that should be sampled (10 or more). The software randomly chose the first box and then gave the coordinates of all boxes needed to be sampled

(that were located in an equal distance from each other). Only those boxes that fell within the cortex (layers I–VI) were included in the measurements. All boxes that fell within the white matter, the pial surface, or neighboring cortical areas were excluded.

Finally, the mean number of neurons per test volume was expanded to total number of neurons per regional volume by the formula

$$N = [\Sigma Q/\Sigma D]XV$$

where ΣQ and ΣV are the summations of the neurons and the volumes of the disectors sampled, and V is the volume of the entire cortical area.

RESULTS

Cytoarchitecture of area 13: qualitative description

In order to identify a structural homologue for the hominoids to the well-described area 13 of the macaque, the investigation of the orbitofrontal cortex in each species was approached in two ways. First, each species was examined independently of the others, and the regions of interest (cortical areas in the posterior parts of the orbital surface) were investigated and defined in relation to the surrounding cortex. Then, parallels were drawn across species, and area 13 was identified on the basis of similar cytoarchitectonic features common in all species investigated. Area 13, although similar, is not identical across species as will be shown here, and its position and structural appearance in relation to that of the surrounding cortex can vary among species.

Human. Most earlier studies of the human orbitofrontal cortex do not recognize a structural homologue to the macaque area 13. Here the cytoarchitectonic features that define area 13 in the rhesus monkey were searched for and were found in the posterior parts of the medial orbital gyrus and the posterior orbital gyrus of the human brain.

Area 13 is bordered medially by the gyrus rectus and laterally by the lateral orbital gyrus and is found in the proximity of the olfactory trigone. The most complete manifestation of the cytoarchitectonic features that define this area can be found in the sections where the medial and posterior orbital gyri tend to merge into one gyrus and the medial orbital sulcus starts to disappear (Fig. 2).

In silver-stained and Nissl-stained histological sections the posterior orbitofrontal cortex has a complex appearance suggesting the presence of several divisions and subdivisions of cortical areas with a relatively short extent. The cortex lateral to the olfactory trigone shows several cytoarchitectonic features that clearly distinguish it from the surrounding cortex. More specifically, layer I has irregular borders with layer II. Layer II is thin and in some sections, incipient, forming clusters of cells rather than a continuous layer. It includes small granular cells that have pale nuclear and cytoplasmic staining. The borders with layer III are hard to define. Layer III is a homogeneous layer that contains medium-sized pyramids. They increase slightly in size toward the borders with IV, but a separation of this layer into sublayers is not as easy as in more rostral parts of the prefrontal cortex. Nevertheless, there is a slight increase in the density of the cells close to the interface with IV. The pyramids in layer III are prominent and have dense nuclear and cytoplasmic staining.

Layer IV is incipient. Its cells are granular and small, with moderate nuclear and pale cytoplasmic staining. Layer V is the most prominent layer and includes darkly stained medium-to-larger pyramids. Most are located in Va. Sublayer Vb and layer VI are poorly segregated. The pyramidal and fusiform cells that are part of Vb and VI have a characteristic horizontal laminar striation, in a direction parallel to the pial surface. Layer VI contains medium-sized pyramidal cells and large fusiform cells that have moderate nuclear and cytoplasmic staining. The boundary with the white matter is not sharp.

In summary, the presence of an incipient layer IV and a medial-to-lateral horizontal striated appearance of the cells in the infragranular layers (V and VI) defines this cortical area. In the surrounding cortex, one or both of the above features is absent. More-rostral cortex is differentiated from area 13 by having a better-defined layer IV that

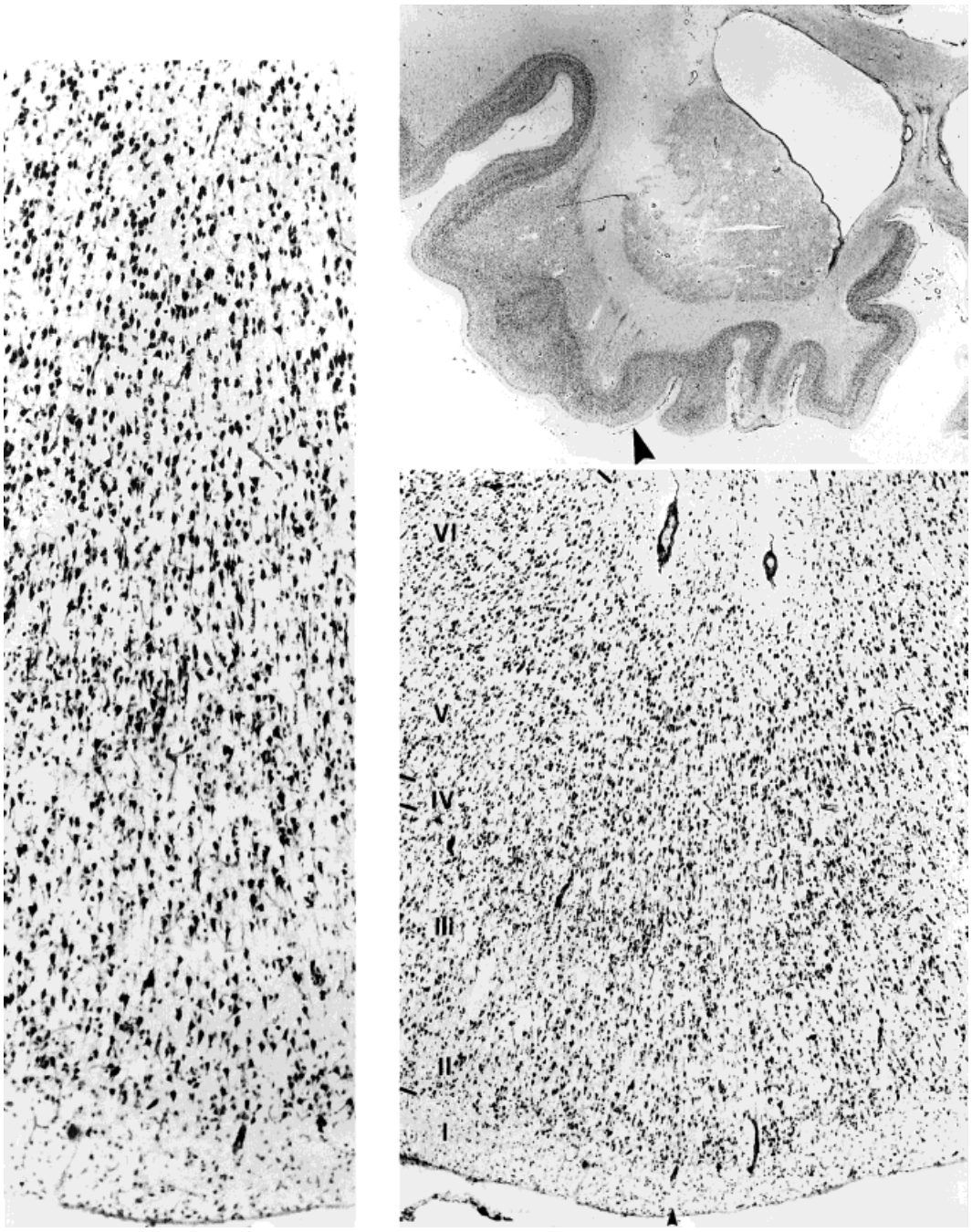

Fig. 2. Photomicrographs of human area 13. The magnifications used are ×2.3 (upper right), ×34 (lower right), and ×62 (left). Large arrowhead marks the presence of area 13 in the upper right corner, and smaller arrowhead marks area of enlargement included in the left. Layers I through VI can be seen and are marked in the lower right photomicrograph.

TABLE 1. Cytoarchitectonic features of area 13[1]

	Human	Chimpanzee	Bonobo	Gorilla	Orangutan	Gibbon	Macaque
Layer I							
Borders	IR	IR	IR	IR	R	IR	IR
Layer II							
Size	IN/TH	TH/M	TH	TH	IN/TH	IN	TH
Cells	S, GR	S, GR	S, GR	S, GR	S, GR	S, GR	S, GR
Staining	PA	PA/MO	DA	MO	MO/DA	MO	DA
Borders	PR	PR	PR	PR	PR	PR	PR
Layer III							
Size	W	W	W	W	W	W	W
Cells	M, PYR	M, PYR	M, PYR	M, PYR	M, PYR	M, PYR	M, PYR
Staining	MO	PA	DA	PA/DA	DA	MO	MO/DA
Borders	VIR	VIR	VIR	VIR	IR	IR	VIR
Layer IV							
Size	IN	IN	IN	IN	IN	IN	IN
Cells	S, GR	S, GR	S, GR	S, GR	S, GR	S, GR	S, GR
Staining	MO	PA	PA/MO	PA	PA/MO	PA	PA
Borders	VIR	VIR	VIR	VIR	IR	IR	VIR
Layer V	Va, b	Va, b	homog.	Va, b	Va, b	Va, b	Va, b
Size	W	W	W	PRO Va	W	PRO Va	W
Cells	M/L PYR	L, PYR	L, PYR	L, PYR	L, PYR	L, PYR	L, PYR
Staining	DA	DA	DA	DA	DA	DA	DA
Borders	PR	PR	PR	PR	CR	PR	PR
Layer VI							
Size	M	W	W	W	W	W	W
Cells	L, PYR, FU	M, PYR, FU	M, PYR, FU	M, PYR, FU	L, PYR, FU	M, PYR, FU	M, PYR, FU
Staining	MO	MO	DA	PA/DA	MO/DA	MO/DA	MO
Borders with white matter	PR	PR	PR	PR	PR	PR	PR

[1] Abbreviations: R, regular; IR, irregular; IN, incipient; TH, thin; M, medium; S, small; L, large; GR, granular; P, pale; MO, moderate; W, wide; PRO, prominent; PYR, pyramidal; DA, dark; VIR, very irregular; PR, poor; FU, fusiform; PA, pale; CR, clear.

cannot be considered incipient. Caudally, the orbitofrontal cortex does not have layer IV, and eventually the cortex disappears.

Medially area 13 is bound by the olfactory trigone and the cortex of the gyrus rectus. The latter exhibits a very different and characteristic structure including a very prominent and darkly stained layer II, and dense but thin layers V and VI. These features are present in the posterior medial orbital gyrus in more-rostral sections. To the lateral side of area 13, the horizontal striated appearance of the cells is completely lost, layer IV is absent, and layer III includes particularly prominent pyramids.

Some of the characteristics of this area vary according to the plane of section and individual variation in the human brains, but the main features characterize the majority of our cases (eight out of ten). The cytoarchitectonic features of area 13 are summarized in Table 1.

Chimpanzee. As in the human brain, the cortex lateral to the olfactory trigone in silver-stained and Nissl-stained histological sections can be distinguished from the surrounding cortex. It includes six cortical layers separated by irregular borders. An incipient layer IV and a prominent medial-to-lateral horizontal (parallel to the pial surface) striated appearance of the cell distribution in layers Vb and VI are the major features of this area. The majority of these are as described for the human and are summarized in Table 1.

Layer II can be better seen in the chimpanzee than in the human cortex. The characteristic parallel striation present in layers V and VI is found in *Pan* as it is in the human and macaque cortex, although it sometimes extends to layer IV in the chimpanzee cortex.

Unlike the human, the features characterizing area 13 were well delineated in all sections. They vary little with the plane of section, and the area is clearly identifiable in all of the chimpanzee series examined. Also, the overall appearance of area 13 in the chimpanzee is less irregular than in the human brains.

Area 13 of the chimpanzee orbital prefrontal cortex is located in the caudal parts of the

medial orbital and posterior orbital gyri. Medially, it is bordered by the gyrus rectus and the olfactory sulcus, and laterally by the orbitofrontal sulcus. As in the human brain, in the chimpanzee area 13 is found in the proximity of the olfactory trigone and is associated with the presence of the claustrum. Unlike the case in the human brain, it appears well before the olfactory trigone and the claustrum, in an anterior–posterior sense. The best manifestation of its cytoarchitectonic features can be found, as in the human brain, in the sections where (in an anterior-posterior direction) the medial and posterior orbital gyri unify into one gyrus and the medial orbital sulcus starts to disappear (Fig. 3).

The chimpanzee orbitofrontal cortex is strikingly similar to the human in the very caudal parts of the medial and posterior orbital gyri. The major cytoarchitectonic features used in defining area 13 in the human apply also to the chimpanzee brain.

Bonobo. This is the first known investigation of the cytoarchitecture of the posterior orbitofrontal cortex of the bonobo brain. The appearance of this entire region is more similar to that of the complex human pattern and less so to that of the chimpanzee (in the sense that it includes several subdivisions of cortical areas). Area 13 is one of several cortical areas present in the bonobo orbitofrontal cortex. The cytoarchitectonic features and the location of area 13 is very similar to that of the chimpanzee brain, as described previously. The characteristic horizontal striated appearance of the cells is present in both layers V and VI (Fig. 4).

Lateral to area 13, the cortex is characterized by the absence of layer IV, whereas the cortex located medially is very thin with a very prominent Va and VIa. Rostrally, there is a clear layer IV in the posterior orbital gyrus, whereas caudally, layer IV is absent.

Gorilla. Area 13 is located in the caudal parts of the medial orbital and posterior orbital gyri in the gorilla. Medially, it is bordered by the gyrus rectus and the olfactory sulcus, and laterally by the orbitofrontal sulcus. As in the human, the chimpanzee, and the bonobo, in the gorilla area 13 is found in the proximity of the olfactory trigone and is associated with the presence of the claustrum. Unlike the case in the human brain, it appears well anterior to the olfactory trigone and the claustrum. Its cytoarchitectonic features can be best appreciated in the sections where the medial and posterior orbital gyri unify into a single gyrus and the medial orbital sulcus disappears.

The microscopic appearance of the gorilla orbitofrontal cortex is strikingly similar to those of the other ape and human cortical areas, especially in the most caudal parts of the medial and posterior orbital gyri. In contrast to the chimpanzee, there is a strikingly prominent layer Va here. The characteristic horizontal striation is present in Vb and layer VI (Fig. 5).

The cortical area lying rostral to area 13 has a clear horizontal striation in layers V and VI in the medial orbital gyrus and some traces of the same horizontal arrangement in the posterior orbital gyrus. Nevertheless, layer IV is clearly not incipient here, but is easily identifiable throughout the extent of the cortex. On the contrary, the area lying caudal to area 13 does not have a layer IV.

Medially, area 13 is bounded by the olfactory sulcus and the gyrus rectus. The cortex here includes a very prominent layer V that is dense and compact. No layer IV is present in the cortex lateral to area 13. The cortex is wider and has a columnar appearance.

Orangutan. Area 13 was present clearly in the orangutan orbitofrontal cortex. Unlike in the other ape and human brains, area 13 in the orangutan brain makes its first appearance in more anterior sections. Thus, the presence of the olfactory trigone is associated only with its more posterior parts. Layer IV is incipient, but it can have a clear presence at some locations. A horizontal appearance of the cells is present in VIa (Fig. 6). The major cytoarchitectonic features present in the other ape and human brains apply to the orangutan brain as well (Table 1).

The cortical area lying rostral to area 13 in the orangutan has a clear layer IV and a more vertical appearance characterized by cell columns. The cortex caudal to area 13 has no layer IV but retains the horizontal striated appearance in the infragranular

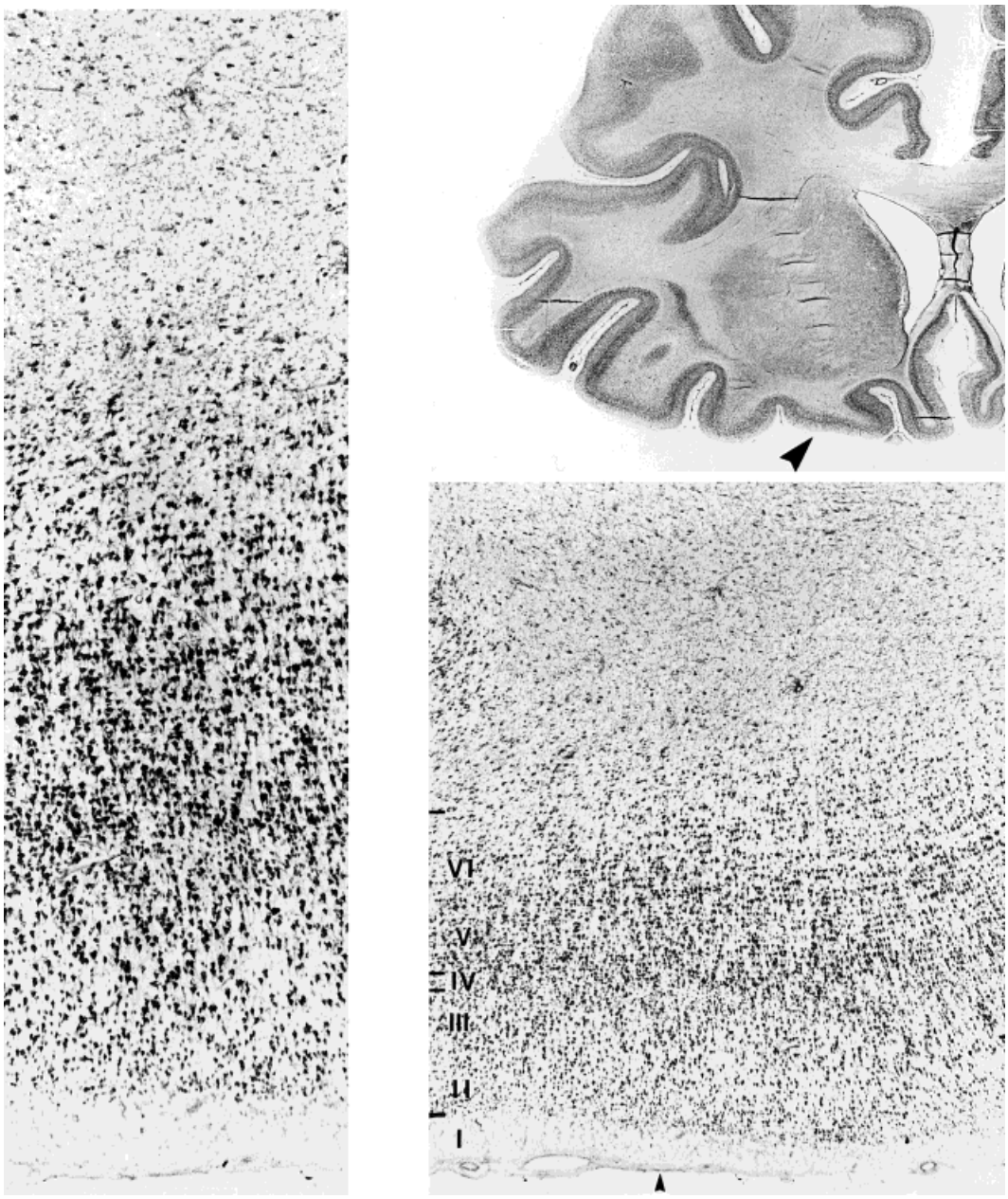

Fig. 3. Photomicrographs of chimpanzee area 13. Magnifications and arrows are as in the human.

layers. Medially (gyrus rectus), the cortex has a very prominent Va and very wide layers I and III. Laterally, the presence of IV is not clear, layer II is prominent, layer IIIa has a low density, and layers V and VI are thin. If homologies are to be maintained, then the above areas would correspond to areas 11, proisocortex, 14, and 12, respectively, of the macaque orbitofrontal cortex (Walker, 1940), but this will remain speculative until the cortex of the apes is investigated.

Gibbon. In the posterior parts of the medial orbital gyrus of the gibbon, layer IV is

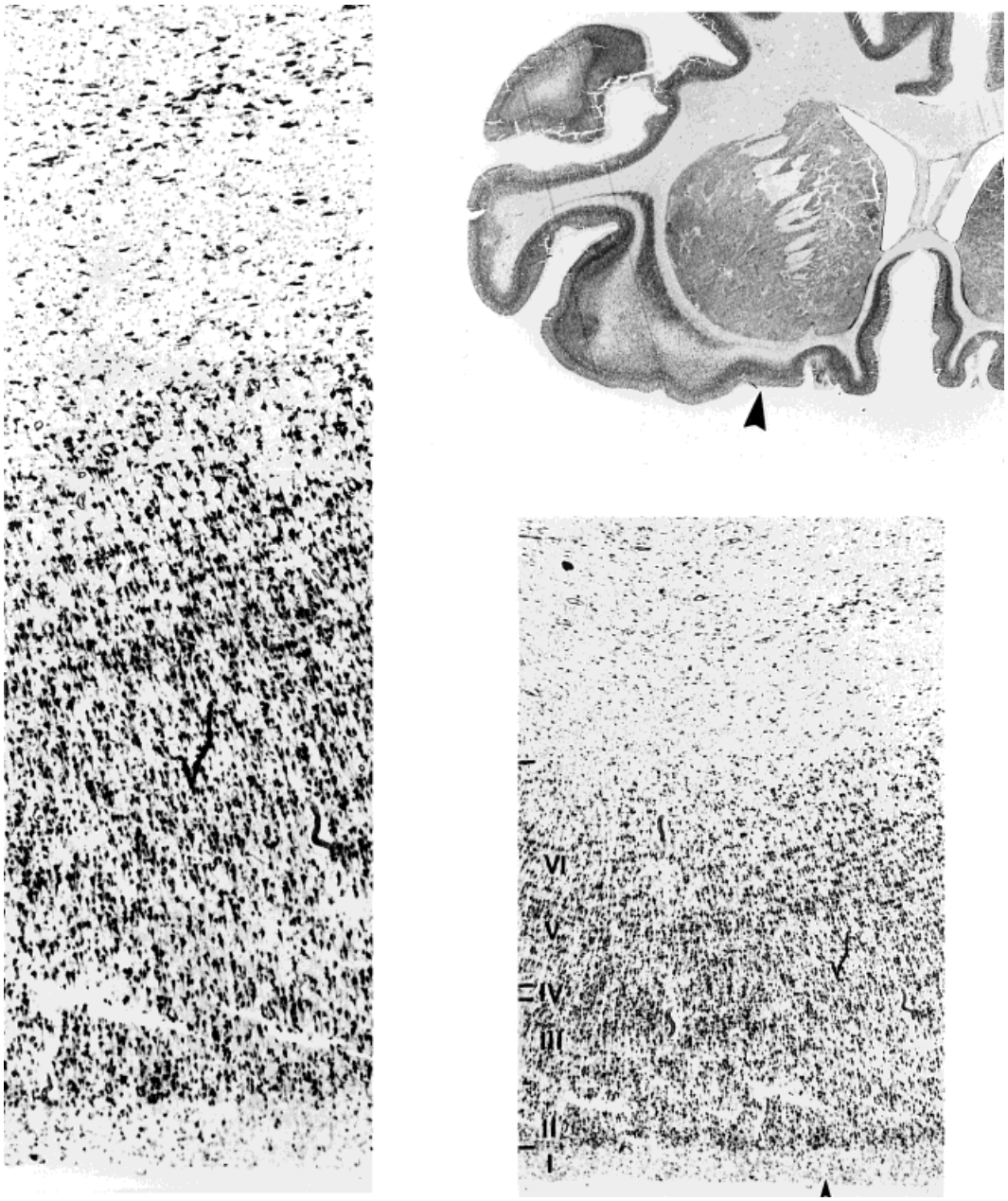

Fig. 4. Photomicrographs of bonobo area 13. Magnifications and arrows are as in the human.

incipient and looks much like layer IV in human area 13. Layer V has a prominent sublayer (Va). In general, area 13 in the gibbon brain has a very irregular appearance, very much like the human brain. Also, similar to the human, the parallel striations in layers V and VI are not as striking as in other apes.

Area 13 in the gibbon orbitofrontal cortex is located in the caudal parts of the medial orbital gyrus. Medially, it is bordered by the gyrus rectus and the olfactory sulcus, and laterally by the orbitofrontal sulcus. As in the human and the other ape brains, area 13 in the gibbon is found in the proximity of the

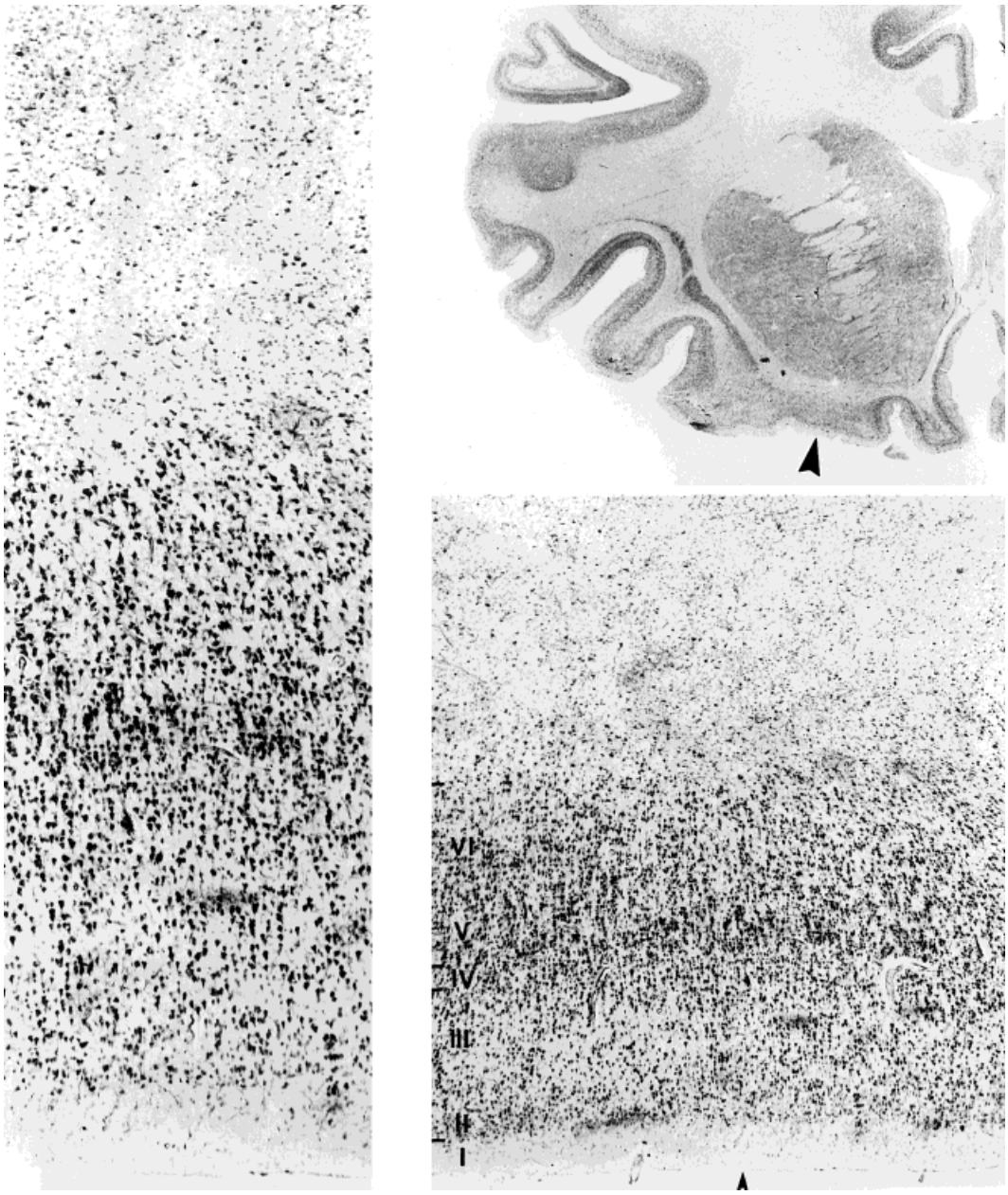

Fig. 5. Photomicrographs of gorilla area 13. Magnifications and arrows are as in the human.

olfactory trigone and is associated with the presence of the claustrum. As in the brains of the other apes, and very much as in the human brain, area 13 of the gibbon brain seems to be restricted to the vicinity of the olfactory trigone. The best example of its cytoarchitectonic features can be found in the sections where the medial orbital sulcus starts to disappear (Fig. 7).

By microscopic evaluation the gibbon orbitofrontal cortex is strikingly similar to that of the other apes and the human cortical area in the very caudal parts of the medial orbital gyrus. The major cytoarchitectonic

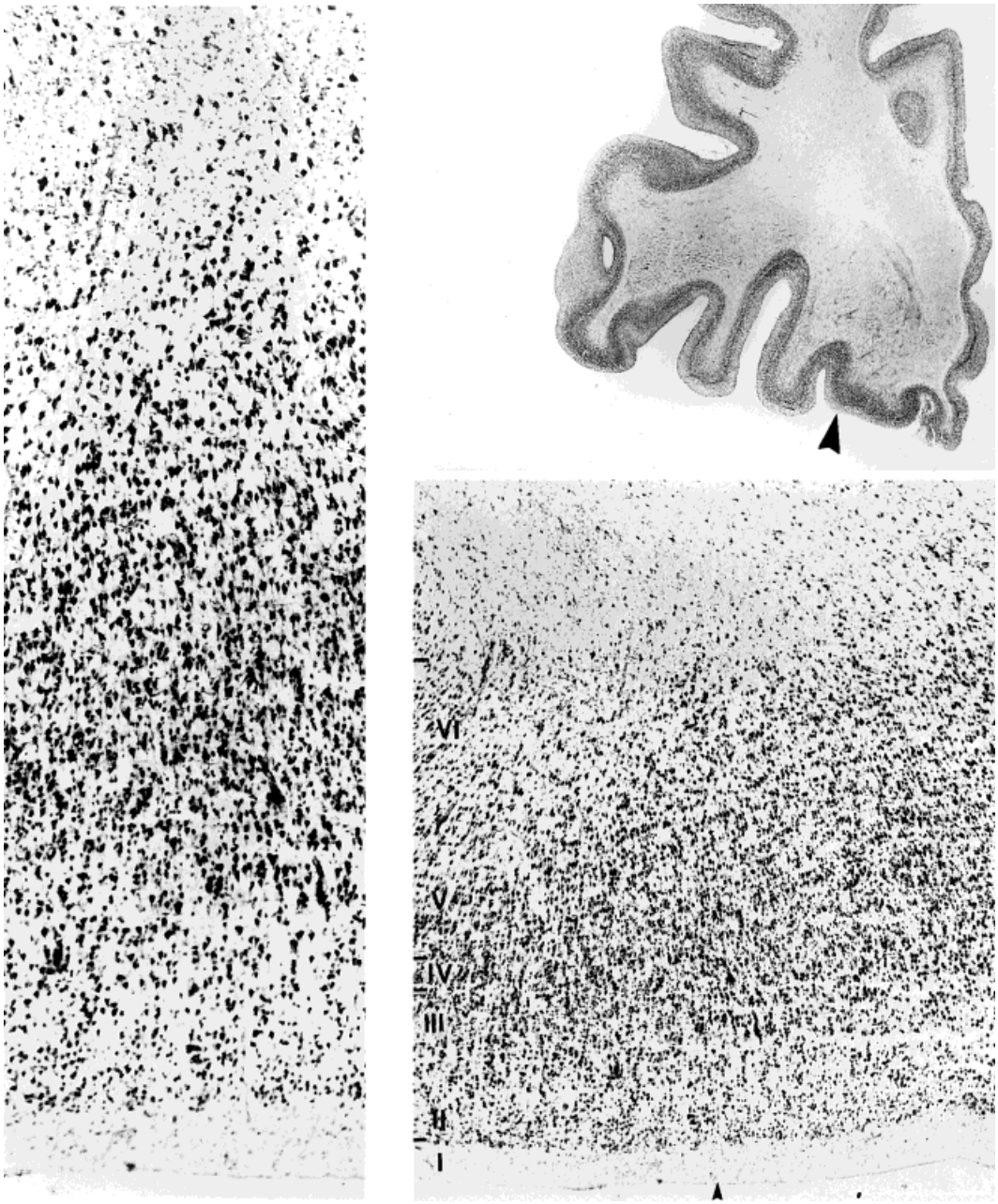

Fig. 6. Photomicrographs of orangutan area 13. Magnifications and arrows are as in the human.

criteria used to define area 13 in the human brain apply nicely to the gibbon brain as well.

Anteriorly, area 13 is bound by cortex that presents some parallel striation in the organization of the cells in layers V and VI, and a clear layer IV. Posteriorly, area 13 is bordered by a cortical type where layer IV is absent. Medially of area 13, the cortex of gyrus rectus includes a very prominent darkly stained Va with large pyramids. Laterally, the cortex has a more prominent

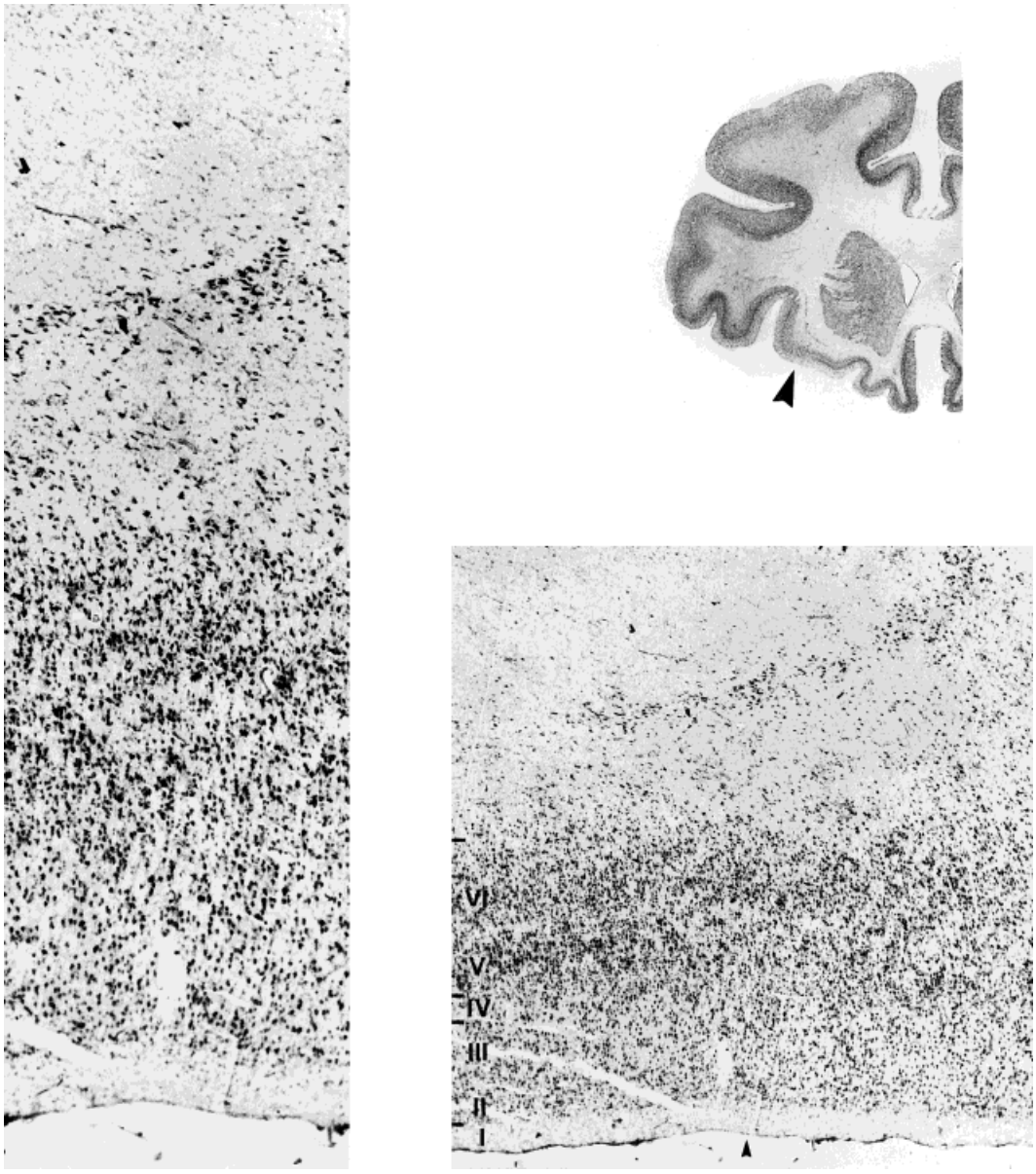

Fig. 7. Photomicrographs of gibbon area 13. Magnifications and arrows are as in the human.

vertical organization, a better-outlined and prominent Va, and a layer IV.

Macaque. Area 13 in the macaque has been extensively described by many researchers. The present analysis agrees with the reports of most other investigators regarding its main characteristics (Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Morecraft et al., 1992; Carmichael and Price, 1994). Layer IV is incipient, although at some locations it has a clear presence similar to the orangutan.

The cortex rostral to area 13 has a distinct layer IV, a very prominent layer V, and a horizontally striated appearance of the cells in layers IV and V, which contrasts with that of area 13. Caudally the above cortex "shifts" medially toward gyrus rectus and area 13

TABLE 2. Volumes of the brain and area 13 in all hominoids[1,2]

Species	Brain	Area 13
Human	1,158,300	366.2
Chimpanzee	393,000	269.9
Bonobo	378,400	110.5
Gorilla	362,900	273.2
Orangutan	356,200	316.6
Gibbon	88,800	51.5

[1] Volume estimates in mm^3.
[2] The figures on the brain refer to total brain structure and on area 13 to measurements in one hemisphere (right).

appears laterally. The cortex caudal to area 13 does not have a layer IV, whereas the cortex lying laterally does. The cortex located medial to area 13 has a clear horizontally striated appearance in the cell layers III, V, and VI. Layer Va is very prominent. The cortex lateral to area 13 has a very clear layer IV (area 12). It should be noted that layer IV in area 13 of the macaque is more "robust" than in the apes and humans, and as a whole, area 13 in the macaque has a more homogeneous appearance than area 13 in the hominoids.

It is clear from a qualitative perspective that the cytoarchitectonic features of area 13 in the well-studied macaque are present in the posterior parts of the orbital cortex of all hominoids. Cortical area 13 is a distinct unit in the medial orbital and posterior orbital gyri of the human, chimpanzee, bonobo, gorilla, and orangutan brain, as well as in the medial orbital gyrus of the gibbon brain.

Size

Total volume of the brain and of area 13, as well as length of the hemisphere, of the orbitofrontal cortex, and of area 13 (in an anterior-posterior direction), were determined for all species.

As expected, the values for the volume of the hemisphere forms three plateaus with the human having the largest value, followed by the four great apes and then the gibbon (Table 2). In contrast, the volume of area 13 has small differences among the hominoids. Human, orangutan, gorilla, and chimpanzee have similar volumes for area 13 in absolute terms (366.2 mm^3, 316.6 mm^3, 273.2 mm^3, and 269.9 mm^3, respectively). The bonobo has a small volume for area 13 (110.5 mm^3) which is less than half the size of the area in the other great apes, whereas area 13 in the gibbon is 51.5 mm^3 (Table 2).

In relative terms human and bonobo values (as a percentage of total brain volume) are similar (Fig. 8), whereas the rest of the great apes form another group, with the gibbon relative value standing in between. This result is very much in line with the qualitative observations that place area 13 and the surrounding posterior orbitofrontal cortex in the human and the bonobo closely together followed by the gibbon.

Allometrically area 13 and brain volume values are not correlated. The size of this area does not increase with brain volume, and values are dispersed.

The values of the total length of the hemisphere, just like those of the volume, form three plateaus (human, great apes, gibbon) (Table 3). The anterior–posterior extent of the orbitofrontal cortex varies among the hominoids (Fig. 9), with the human having the largest value (44.5 mm) followed by the gorilla (36 mm), the chimpanzee and the bonobo (both 30 mm long), the orangutan (24 mm), and then the gibbon (18 mm). The anterior-posterior extent of area 13 is similar in the human and the bonobo (3 mm and 3.5 mm respectively). It is also similar in the chimpanzee, gorilla and orangutan (5.6 mm, 5.4 mm and 5.4 mm, respectively), and smallest in the gibbon (2.2 mm).

In relative terms the human has the smallest anterior–posterior extent of area 13 within the posterior parts of the orbitofrontal cortex (7% of the length of the orbitofrontal cortex), followed by the bonobo and the gibbon (both 12%). The gorilla (15%) and the chimpanzee (19%) follow. The orangutan has a very short orbitofrontal cortex (2.4 cm vs. 3.6 cm in the gorilla), contributing in a relatively larger extent of area 13 within the orbitofrontal cortex than in the other apes (it extends in 23% of the total length of the orbitofrontal cortex in an anterior–posterior direction). This ape presents a unique case with a smaller orbitofrontal cortex largely occupied by a single cortical area.

Quantitative signature

Grey-level index (GLI). Selected sections were measured in order to estimate the

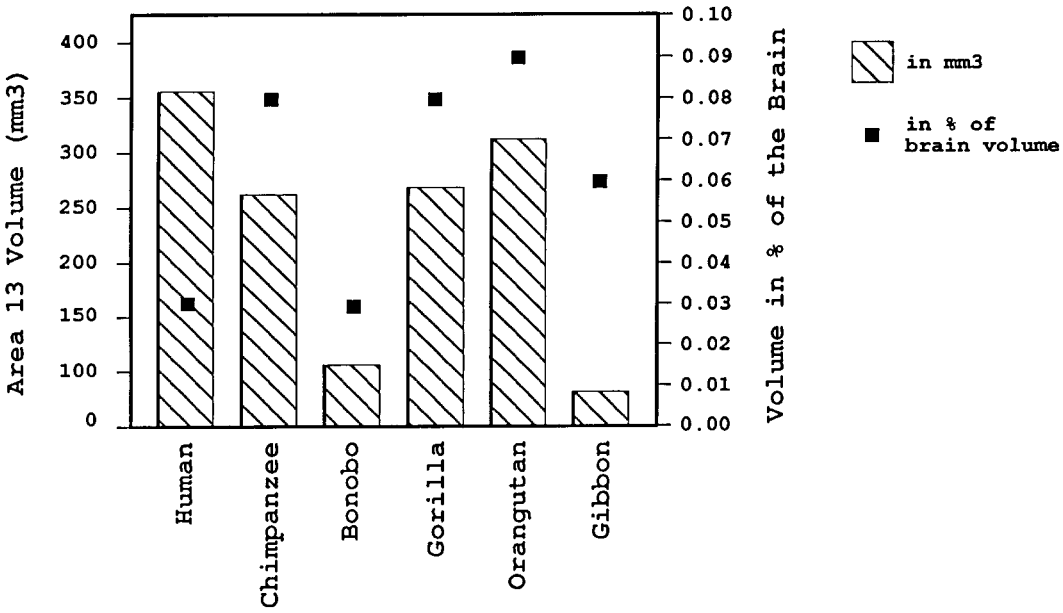

Fig. 8. Absolute and relative size of area 13.

TABLE 3. Length of the right hemisphere, orbitofrontal cortex, and area 13 (in mm)

Species	Hemisphere	Orbitofrontal cortex	Area 13
Human	151.5	44.5	3.0
Chimpanzee	85.0	30.0	5.6
Bonobo	90.0	30.0	3.5
Gorilla	97.0	36.0	5.4
Orangutan	91.0	24.0	5.4
Gibbon	55.0	18.0	2.2

areal percentage of the cortex covered by stained particles versus neuropil space. High GLI values indicate increased space occupied by cell bodies, whereas lower GLI values may suggest increased space filled with neural processes, such as dendrites and axons (Schlaug et al., 1993).

The mean values for area 13, including all cortical layers (II through VI), as well as the mean value for the supragranular (or outer) layers (II and III), granular layer IV, and infragranular layers (V and VI) are given in Table 4.

The lowest mean GLI value for area 13 is that of the gibbon brain (13.33), followed immediately by the human and the gorilla (14.18 and 14.62, respectively). Next come the bonobo (16.98), macaque (18.36), orangutan (18.55), and chimpanzee (18.63). The distribution of the values among the layers is as follows: The granular layer (layer IV) has in every species the highest GLI value (ranging from 15 to 20), whereas supragranular values are somewhat lower than the values in the infragranular layers in most species, with the exception of the bonobo and the macaque, in which values are similar in the supra- and infragranular layers.

The small differences in the GLI values between the supra- and infragranular layers is in agreement with the qualitative analysis of the microscopic sections, where no major differences were observed in cell size or cell density throughout the depth of the cortex in area 13. Nevertheless the small differences (infragranular layers reach slightly higher values than the supragranular layers) suggest that the infragranular layers are more densely populated or include larger neurons than the supragranular ones.

The variation in the percentage of space occupied by cell bodies versus neuropil space can be illustrated in the form of profiles (Fig. 10). In such a profile the x-axis represents the cortical depth and the y-axis represents the GLI values. The profiles start at the pial surface and extend to the border of layer VI with the white matter.

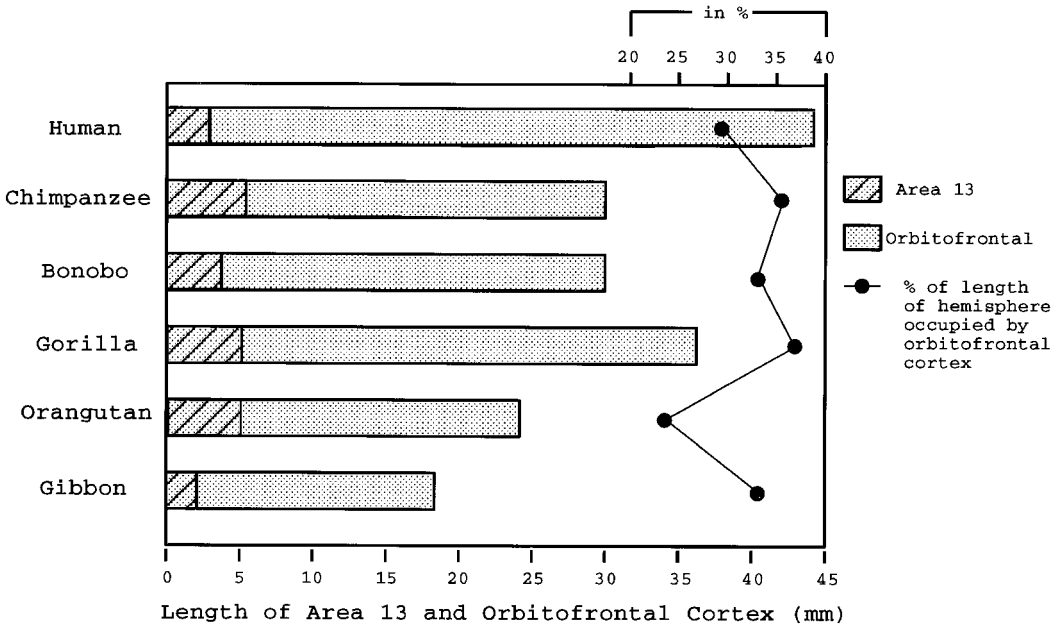

Fig. 9. Extent of area 13 and the orbitofrontal cortex in an anterior–posterior direction.

TABLE 4. Grey-Level Index in area 13[1]

	Mean	StDev	Layers	Mean	Average standard deviation
Human	14.18	1.2898	L II, III	13.79	1.2523
			L IV	15.09	1.4133
			L V, VI	14.13	1.6680
Chimpanzee	18.63	1.5534	L II, III	17.60	1.6308
			L IV	19.37	0.4601
			L V, VI	18.93	1.8171
Bonobo	16.98	2.4222	L II, III	16.66	2.2067
			L IV	18.05	1.1332
			L V, VI	16.67	2.8779
Gorilla	14.62	2.2562	L II, III	12.89	2.0245
			L IV	16.71	0.4613
			L V, VI	15.32	2.3750
Orangutan	18.55	1.1185	L II, III	17.90	2.1738
			L IV	19.68	0.3295
			L V, VI	18.06	1.8529
Gibbon	13.33	2.1650	L II, III	11.61	1.4600
			L IV	15.94	1.0521
			L V, VI	13.82	2.9277
Macaque	18.36	1.6833	L II, III	18.11	1.6899
			L IV	18.73	0.6068
			L V, VI	18.21	2.1988

[1] Measurements were taken from the right hemisphere in each species.

The human profile for area 13 shows a relative homogeneity in the overall distribution of the GLI values. The peaks in the profile correspond in parts of layer V and III, which as discussed previously, include large pyramidal cells (especially in IIIb and Va). Lower values in the profile include the upper parts of layer III and VIb as well.

Compared to the human, the chimpanzee area 13 profile is characterized by an even higher degree of homogeneity in the distribution of values (Fig. 10) and is bimodal. There are two plateaus, rather than peaks, with higher values in layers II/IIIa and Va. The bonobo profile indicates less homogeneity in the distribution of the GLI values than that of the chimpanzee. There are some lower values within the upper parts of layer III as well as just before the occurence of layer IV. The profile of gorilla area 13 shows significantly lower values for the supragranular layers. The lowest values are found in the upper parts of layer III. In the orangutan the distribution of values creates a multimodal shape in the profile with a significant minimum within the borders of layer IIIa. In contrast, the distribution of the values in the gibbon creates a bimodal profile with considerably higher values in the infragranular layers. The gibbon profile is split into two GLI levels. The lower one consists of layers II and III, whereas the higher one includes layer IV, V, and VI. It has a unique shape and an overall very low mean GLI value (13)—the lowest of all species studied. The

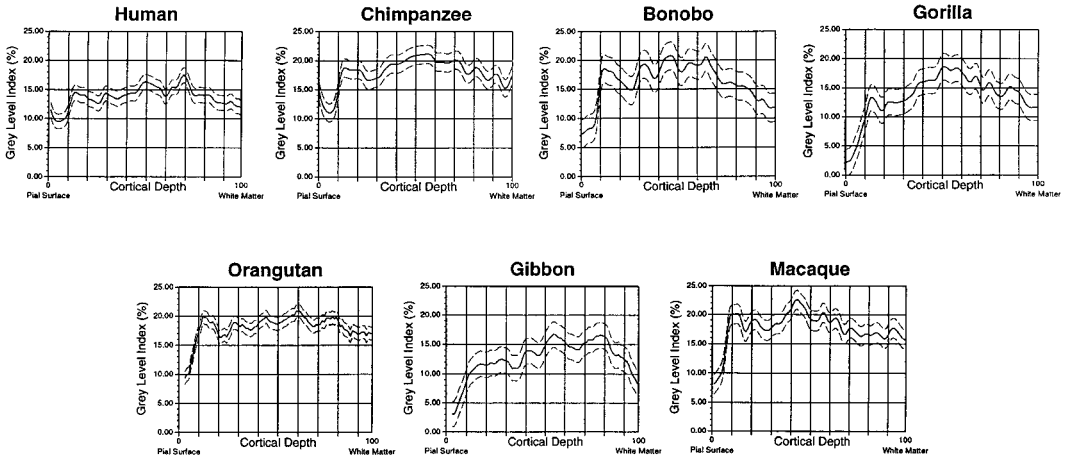

Fig. 10. Mean Grey-Level Index (GLI) profiles of the human, chimpanzee, bonobo, and gorilla (upper row) and the orangutan, gibbon, and macaque (lower row). Peaks in the profiles represent areas covered more by cell bodies, leaving less space available for connections.

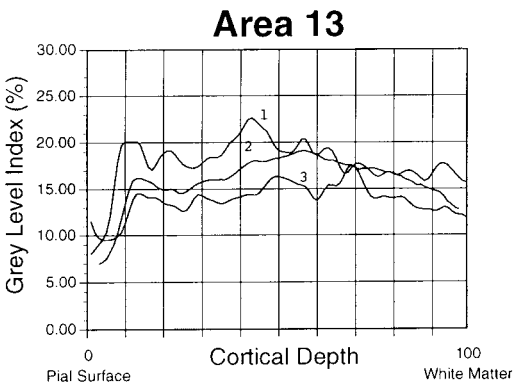

Fig. 11. Comparative plots of GLI profiles in area 13 of the human (3), ape (2), and macaque (1).

profile of the macaque area 13 shows a relatively homogeneous distribution of values. There are no observable major peaks or lows.

In general terms, the human profile has lower values than those of the great apes (Fig. 11), the gibbon has the lowest values, and the macaque the highest. The overall distribution of the values, as reflected by the overall shape of the human and ape profiles, is very similar, in particular the first 45% of the cortical depth, which corresponds to supragranular layers involved in short (layer II) and long (layer III) association connections.

Relative size of cortical layers. One of the most prominent features of area 13 is the fact that the infragranular layers (V and VI) are particularly wide, occupying a large percentage of the total cortical depth. This was clearly observable in the qualitative evaluation and is here documented in terms of the percentage of the cortical layers to the depth of the cortex (Fig. 12). Across species, the supragranular layers are thinner than the infragranular layers, ranging from 28% to 43% and 44% to 59%, respectively. Layer IV is thinnest in the African ape/human group (3–3.5%), but wider in the orangutan, gibbon, and the macaque (6%).

Neuronal numbers. The density of neurons was estimated throughout the depth of the cortex in all species (Table 5). As expected, the lowest density is to be found in the human brain (30,351 neurons/mm^3) followed by the orangutan (42,400 neurons/mm^3) and the bonobo (44,111 neurons/mm^3). Higher densities are observed in the chimpanzee, gorilla, and gibbon (50,686, 54,783, and 53,830 neurons/mm^3, respectively).

Total numbers of neurons are highest in the gorilla, orangutan, chimpanzee, and human, ranging from 7.5 million to 5.85 million neurons. The bonobo and the gibbon have smaller numbers (2 million and 1.3 million, respectively), which is due to the small volume of area 13.

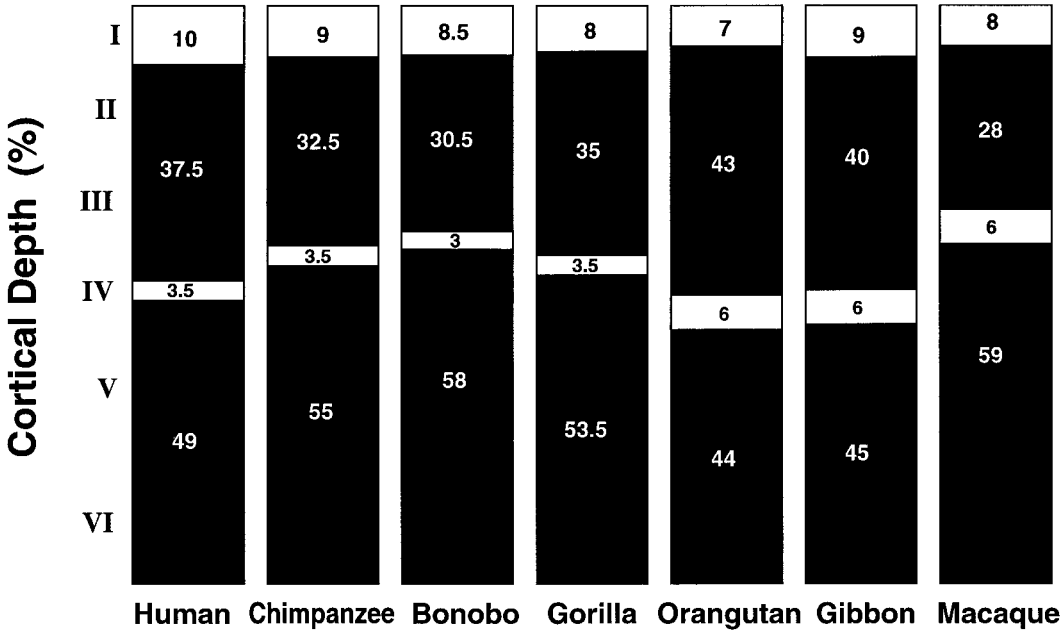

Fig. 12. Relative width of cortical layers. The columns start with the most superficial layer, layer I (at the top), and continue through the depth of the cortex to layer VI, which is bordering the white matter.

TABLE 5. Total numbers of neurons and neuronal density (per mm³) in area 13[1]

Species	Total numbers	Density of neurons	SE
Human	5,850,000	30,351	2,800
Chimpanzee	6,500,000	50,686	3,500
Bonobo	2,000,000	44,111	3,300
Gorilla	7,500,000	54,783	4,250
Orangutan	6,700,000	42,400	3,600
Gibbon	1,300,000	53,830	4,100

[1]Estimates refer to one hemisphere (right) per species.

DISCUSSION

Previous qualitative analyses of the cytoarchitecture of the orbitofrontal cortex have involved largely selected monkey species, usually the rhesus monkey (Walker, 1940; Bonin and Bailey, 1947; Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Morecraft et al., 1992; Carmichael and Price, 1994), whereas older studies addressed the human cortex as well (Brodmann, 1909; Economo and Koskinas, 1925; Kononova, 1949, 1955; Bailey and Bonin, 1951; Sarkissov et al., 1955; Sanides, 1962). With the exception of few attempts to analyze the chimpanzee cortex (Bailey et al., 1950), the cortex of the apes has remained virtually unknown.

Cytoarchitectonic variations provide a large amount of information on the topography of the cortex and have been used traditionally to generate maps. They form a necessary first step to describe the cortex and identify possible homologies. Examination of the chimpanzee, bonobo, gorilla, orangutan, and gibbon brains is of particular relevance and importance in attempting to relate the well-studied cortex of several monkey species to that of the human cortex. Considering the very close relationship that humans share with the apes, one would expect that much information would be available on the cortex of our closest relatives. Surprisingly, very little is known about the cortex of the apes.

Quantitative morphological studies of various areas of the brain (for example, on the neocortex or posterior cingulate cortex) have shown in the past that there are differences in aspects of the organization of the brain in primates (Zilles et al., 1986; Stephan et al., 1981; Armstrong, 1980). Furthermore, recent applications of quantitative criteria in the mapping of the human (Rajkowska and Goldman-Rakic, 1995a,b) as well as human and non-human primate cortex (Armstrong

et al., 1986) have demonstrated that the delineation and identification of cortical areas can be assisted substantially by the use of morphometric techniques that are reliable and reproducible. In this study, we identified an area of the frontal lobe, area 13, in humans and apes using qualitative and quantitative criteria. We also investigated this area in the macaque monkey in order to relate the information to the vast amount of knowledge available today from experimental studies on the rhesus monkey.

Cytoarchitecture of area 13 and comparisons with previous studies

Area 13 has been known to be part of the orbitofrontal cortex in the rhesus monkey for several decades. Walker (1940) located it on the posterior part of the orbital gyrus lateral to the olfactory trigone and described its cortex as fairly wide, with a much fainter inner granular layer, which is more loosely arranged than that in the area lying rostrally (area 11). Bonin and Bailey (1947) refer to the macaque posterior orbital cortex as "FF," a cortical type that includes an "indistinct layer IV," a thin layer II, and a wide layer VI. More recently, the organization and connections of area 13 were addressed in monkey species (Van Hoesen et al., 1972, 1975; Van Hoesen, 1981). Barbas and Pandya (1989) point out that this region resembles the caudally situated proisocortex, but has a slightly higher cell density in layer III when compared to the proisocortex. Moreover, there is an incipient layer IV consisting of occasional granular cells. Like the proisocortex, area 13 also has slightly more prominent deep layers, with layer V being the most prominent. Preuss and Goldman-Rakic (1991) found that areas 13, 14, and their subdivisions make up the largest part of the orbital surface in the rhesus monkey. They suggest that the cortex of area 13 should be divided into medial and lateral subdivisions, occupying the orbital sulcus (13M) and the convexity lateral to the sulcus (13L). In Nissl stains, layer IV is present in both 13L and 13M as an irregular band of loosely arranged cells. Morecraft et al. (1992) mapped the orbitofrontal cortex of the macaque using cytoarchitectonic criteria and connectional studies. They include Walker's area 13 in the orbital dysgranular cortex (OFdg) and describe it as including an emergent layer IV, which consists of a thin sheet of granule cells occasionally interrupted by the incursion of large pyramidal cells. In the most recent study of the macaque prefrontal cortex, Carmichael and Price (1994) apply various histochemical and immunohistochemical stains and remap this part of the brain. A correlation of Nissl, myelin, AChE, calbindin, SMI-32, and 8b3 antibody staining lead them to subdivide area 13 into 13m, 13l, 13a, and 13b.

Area 13 is absent from Brodmann (1909), the widely used map of the human cortex. A review of the literature nevertheless reveals that this seemingly ignored area was recognized under different designations in most other studies. As Rajkowska and Goldman-Rakic (1995a) point out, "neuroscientists have tended to rely almost exclusively on Brodmann's map, even though this map is based on only one case and is not accompanied by a description of the criteria used for distinguishing the areas charted or by photographic illustrations."

Even Brodmann (1909), who divides the human orbitofrontal cortex into areas 11 and 47, comments that "there are many more subdivisions within area 11 (e.g., area orbitalis interna)." He nevertheless does not designate any area 13 in the human cortex nor, as a matter of fact on his *Cercopithecus* map. It is Walker who first introduces area 13 in the map of the macaque cortex and also comments (1940) about Brodmann's mapping of the human orbital cortex: "Brodmann (1909) does not designate a corresponding division to this area (13) in either the human or macaque brain. It is possible that this field might correspond to a zone, which he states can be separated from area 11, and which he terms area orbitalis interna."

Economo and Koskinas (1925) discuss in detail their parcellation of the human orbitofrontal cortex. "The orbital cortex has a fluid architecture, that changes from one millimeter to the other. The cells of layer IV become more slender and this layer as a whole loses its significance towards the caudal parts. It becomes thinner towards the end of the orbitofrontal cortex and in some brains it

eventually disappears and FF seems to be agranular in these cases" (1925).

Bailey and Bonin (1951) state that "coming from subhuman primates we are more impressed by the astounding homogeneity of the human cortex than by its divisibility into a host of elemental organs as Brodmann would have it." Throughout their book they criticize previous attempts to map the human brain and they break down the human isocortex into only a few major cortical types. At the same time, Bailey and Bonin recognize two prominent trends present in the orbitofrontal cortex: "the gradual disappearance of the inner granular layer as the posterior margin of the orbital surface is approached, and the gradual appearance of allocortical characteristics as the gyrus rectus is approached. The overlapping of these two tendencies make possible numerous subdivisions." It is of interest to note that even von Bonin and Bailey, who severely criticize others' attempts for multiple subdivisions of the cortex, admit to the following: "E. Beck has some justification for extending Brodmann's area 13, which that author recognized in Hapale and Lemur, to the posterior zone of the orbital cortex" (1951).

Beck (1949) compared the posterior parts of the orbitofrontal cortex in the macaque and in humans. She found a clear structural homologue to the macaque area 13 in the human cortex, and furthermore states that "area 13 in man is far more restricted . . ." and "can be termed an agranular subdivision of area 11. It retains all the main characteristics of area 11, including a very marked layer Va with especially large cells towards the posterior end and a well-developed layer Vla. The latter two features give rise to distinct horizontal striation, which also constitutes the main difference from area posterior. The transition from granular to agranular cortex is gradual, but extends only over 100 sections as compared with 200 on gyrus rectus" (Beck, 1949).

Sanides (1964), who mapped the human frontal lobes, includes the posterior parts of the orbitofrontal cortex within what he names FoZ. He notices "a stepwise granularization and stronger lamination as well as diminishing of V pyramids with an increasing accentuation of IIIc pyramids and decreasing overall cell size at the same time" (1964), as one moves from the most caudal parts of the orbital cortex toward the frontal pole. Furthermore, "The caudal fields of the orbital cortex reveal the rhinencephalic influence, moreover, in the less pronounced layering and fainter granularization. This is consistent with the cytoarchitectonic observations of Beck (1949)."

It is obvious from the above brief review of the literature that most researchers agree that area 13 is part of the orbital surface of the human frontal lobes, which is in agreement with the findings presented here.

Regarding the cortex of the apes, it is only Bailey et al. (1950) who make a comparative cytoarchitectonic analysis of the chimpanzee cortex. In their discussion of the orbitofrontal cortex, they include areas FE, FF, and FG. In FE they demarcate the orbital part of the frontal pole area that, according to their figures, occupies the most rostral one-third of the total length of the orbital surface. FG represents the cortex of the gyrus rectus, and FF occupies most of the medial and posterior orbital gyri. They describe FF as including an irregular lamination, tenuous layer IV, and a blurred boundary with the white matter. Again, our findings on the chimpanzee cortex are in agreement with the above descriptions.

Based on silver and Nissl stains, this study of cortical cytoarchitecture establishes that the well-studied macaque area 13 has a structural homologue in the brains of the gibbon, orangutan, gorilla, bonobo, chimpanzee, and human. The observations made here on the cytoarchitecture of this part of the orbitofrontal cortex in the human brain are in agreement with most of the older studies, as presented above, that describe a similar cortical organization under a variety of names.

Hominoid area 13 is located in the posterior parts of the medial orbital and posterior orbital gyri of the orbitofrontal cortex (Fig. 13). The cortex in area 13 is characterized by the presence of an incipient layer IV and a medial-to-lateral horizontally striated appearance of the cells in layers V and VI. Other microscopic features present in all species studied are the large size of the pyramidal cells in layer V, the thickness of

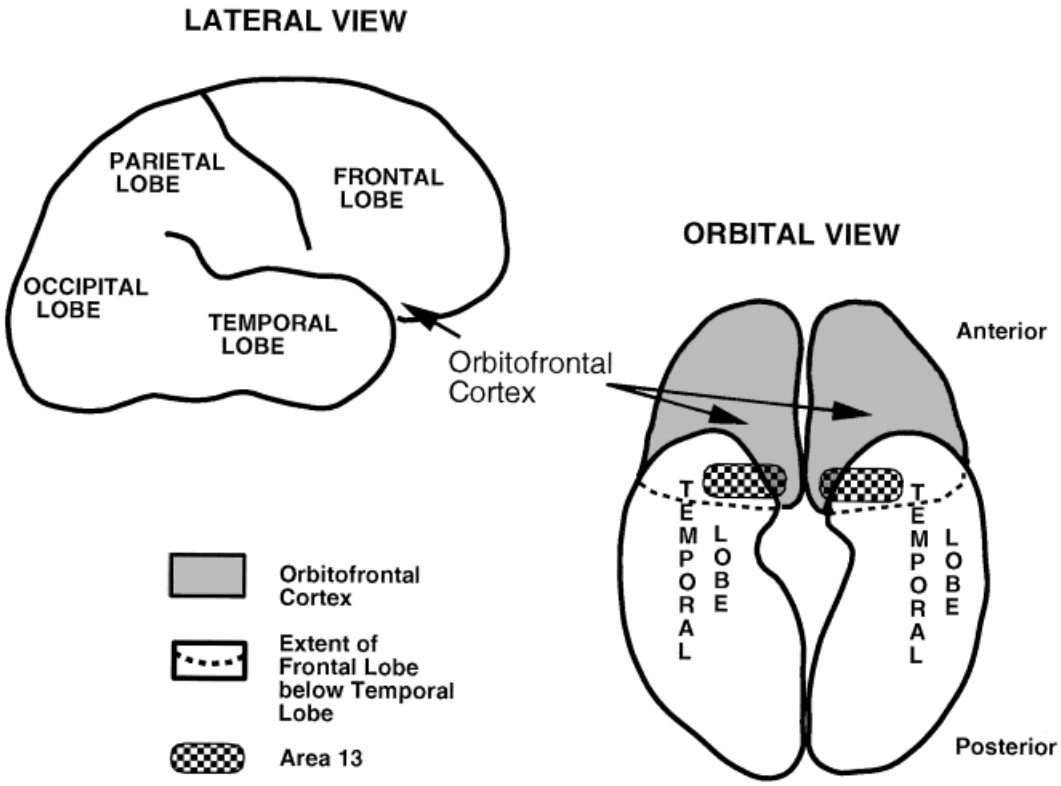

Fig. 13. Graphic representation of the approximate location and extent of area 13 in the orbitofrontal cortex of a hominoid brain.

the infragranular layers (V and VI) in comparison to the supragranular layers (II and III), and the larger neuropil space in the supragranular versus the infragranular layers.

In all species studied, one major criterion for differentiating area 13 from the more rostrally located cortical areas (whether the area is 11, or a transitional area, or a subdivision of 13) is the fact that layer IV is incipient. When this layer becomes more robust and shows a more uniform cell distribution, then that cortex does not belong to area 13, but to the neighboring cortical area.

Quantification of area 13 across hominoids

In addition to the above qualitative features, area 13 is characterized by quantitative relationships of some of its structural features. At this point it is necessary to emphasize that the results of the quantification need to be viewed as preliminary due to the small sample used. The present study, although more inclusive than any previous comparative study of the hominoid prefrontal cortex, cannot address the possible significance of intraspecific variation.

Area 13 GLI values in the supragranular, granular, and infragranular layers are consistent (Table 4). Values of layers II and III are lower than those of layers V and VI in each species. Granular layer IV has the highest value. These results indicate increased neuropil space in the supragranular layers. It is known that layers II and III are involved in short and long association connections, and lower GLI values may suggest increased space filled with postsynaptic sites, such as dendrites. The distribution of the GLI values is also very similar in the profiles of the apes and human, especially in the overall shape through a standardized corti-

cal depth, suggesting a similar distribution of cell types and/or cell sizes.

All hominoids have a similar ratio in width of cortical layers (Fig. 12). In all the species, the infragranular layers are larger than the supragranular layers. Layer IV is thinner in the African ape/human group (3–3.5%) and slightly thicker in the orangutan, gibbon, and the macaque (6%).

The neuronal density in area 13 is the lowest in the human (30,351 neurons/mm^3), followed by the orangutan (42,400 neurons/mm^3), the bonobo (44,111 neurons/mm^3), the chimpanzee (50,686 neurons/mm^3), the gibbon (53,830 neurons/mm^3), and the gorilla (54,783 neurons/mm^3). The human value is considerably smaller than the ape values, and the density of neurons in area 13 correlates with brain volume ($r = 0.87$). A high density of neurons is observed, as expected, in the small-sized gibbon brain.

The absolute number of neurons in area 13 is the largest in the gorilla (7.5 million), followed by the orangutan (6.7 million), the chimpanzee (6.5 million), the human (5.9 million), the bonobo (2 million), and the gibbon (1.3 million). Because the absolute number of neurons is a function of total volume, the low total number of neurons in the bonobo is attributed to the small size of area 13. The similarity of the numbers in the human and the other great apes is attributed to similar size as well.

The total volume of area 13 in the right hemisphere of the great apes and humans is very similar, ranging from 269.9 mm^3 in the chimpanzee to 366.2 mm^3 in the human (Table 2). The only noticeable exception among the great apes is the bonobo, with 110.5 mm^3. In this species, the difference in the volume of the area should be attributed to its restricted relative length (present only in a few millimeters of the posterior orbital cortex, as will be discussed).

The relative (to the entire brain) volume of area 13 is very small in humans (0.03%) and bonobos (0.03%) (Fig. 8). The gibbon has a medium relative value (0.06%). The orangutan with the highest (0.09%) relative value is close to that of the gorilla and the chimpanzee (0.08%).

Another measure of the size of area 13 is its extent in an anterior–posterior direction. Table 3 includes the absolute values for the length of area 13, and Figure 9 reflects the relative values for its presence in the length of the hemisphere and the orbitofrontal cortex. Area 13 in the gibbon has the smallest length (2.2 mm), followed by the human (3 mm) and the bonobo (3.5 mm). Area 13 is more extended in the macaque (5.8 mm), the chimpanzee (5.6 mm), the orangutan, and the gorilla (5.4 mm in both apes) (Fig. 9). Obviously its anterior–posterior extent is independent of brain size.

In relation to the length of the orbitofrontal cortex, area 13 in the human brain is present in only 7% of its length, whereas in the orangutan it is present in 23% (largest relative values among the apes). In relative terms, area 13 of the human brain is present in only 2% of the total length of the hemisphere; in the apes it is present in 3.9% to 5.9%. The homogeneity in the distribution of the volume values does not match the relative size of the area within the orbitofrontal cortex.

In the human brain, the overall volume of area 13 is larger than that in the orangutan brain (404.8 mm^3 versus 316.6 mm^3), but it extends for only 3 mm in the orbitofrontal cortex versus 5.4 mm in the orangutan. This indicates that the difference in size of this area is due to a medial to lateral expansion of the orbitofrontal cortex (that includes area 13) along with the overall increased size of the entire brain, rather than an expansion in an anterior–posterior direction. If we take into consideration that layer IV becomes more robust in more rostral parts of the orbitofrontal cortex, then the findings indicate that there may be an increased number of cortical areas or subdivisions in the human orbitofrontal cortex. The same might be true for the bonobo as well.

Three of the great apes (orangutan, gorilla, and the chimpanzee) have large values for area 13, while the gibbon, bonobo, and human have small areas 13 (Fig. 9). This indicates that the gibbon, bonobo, and human orbitofrontal cortex have smaller areas 13 than would be expected from a hominoid of their brain size. A larger sample number is desirable, but regarding the uniqueness in the size and organization of the orbitofrontal sector of the orangutan, this was noticed

in several specimens and is thus less likely to change with the addition of more specimens.

CONCLUSIONS

The presence of cortical area 13 has long been recognized in the macaque brain (Walker, 1940). Although it was largely ignored in the classical parcellations of the cortex (Brodmann, 1909), some early studies have also acknowledged its distinct features in the human brain (Beck, 1949). The present study reveals that humans, great apes (*Pan troglodytes, Pan paniscus, Gorilla gorilla, Pongo pygmaeus*), and lesser apes (*Hylobates lar*) have a distinct area 13 located in the posterior parts of their orbitofrontal cortex.

In all hominoids this cortical area is characterized by the presence of several common cytoarchitectonic features that set it apart from the surrounding cortex. These features and the relationships between them as described and compared in qualitative and quantitative terms point to an overall similar structural organization of area 13 across species. Nevertheless, minor differences in its structure are clearly present, and there is a degree of interspecific variation in the relative size of area 13.

The orangutan has a unique organization in the orbital sector of the frontal lobe. This includes a smaller overall size for this sector in both absolute and relative terms than in any other hominoid, a more homogeneous orbital sector with a single cortical area occupying a relatively larger segment of it, and a limbic area (area 13) that is "less limbic" in its organization in comparison to the other great apes (it has a wider granular layer more typical of prefrontal association cortices than frontal limbic cortices).

Recent evidence from lesion studies in humans associates the orbital and mesial frontal sector, the limbic frontal cortex, with a variety of deficits in behaviors in the social domain (frontal lobe damage causes sociopathic behavior, insensitivity to future consequences, drastic changes in personality). It is reasonable to believe that this sector is important for the survival of members of complex social groups. The orangutans have a smaller and more homogeneous frontal limbic cortex. Orangutans are also known to have a more solitary life-style and less complex social organization than the rest of the hominoids.

In contrast, it was shown here that humans and bonobos have a relatively smaller area 13 and a complex organization in the surrounding orbitofrontal cortices that might suggest the presence of a larger number of cortical subdivisions in these species. It is tempting to associate this finding with the well-known complex social and emotional behaviors of humans and bonobos, although such a finding is hard to reconcile with the fact that chimpanzees have complex societies as well. Given the small sample size in the case of the bonobos (an outlier?), it is imperative that more cortical areas are investigated in a larger number of individuals before any firm conclusions can be reached.

Area 13 has been known to be associated with changes in emotional states and disinhibition of emotional reactions in the macaque. Removal of this cortex has been found to enhance aversive reactions and reduce aggressive reactions in several threatening situations. These emotional alterations have been interpreted on the basis of the close relationships that the posterior orbital cortex has with the limbic structures, especially the mediodorsal nucleus of the thalamus and the amygdala (Butter and Snyder, 1972). Studies performed in the wild, as well as in laboratory social settings, on monkeys with orbitofrontal ablations have shown significant reductions and losses of behaviors that are considered important for the maintenance of social bonds (Kling and Steklis, 1976). Studies of social cognition among primates (De Waal and Aureli, 1996; Ingmanson, 1996; Byrne, 1996; Van Schaik and Van Hooff, 1996) emphasize species-specific patterns in the way that individuals deal with conspecifics and the importance of this behavior for the survival and reproduction.

Cytoarchitectonic qualitative evaluations do not provide a complete account of homology between cortical areas. Nevertheless, examination of several species with the same technique in a consistent manner is the necessary first step, but unfortunately is rarely attempted. The quantification of the cortex stained with conventional techniques,

as well as studies involving connections, myelin, and immunohistological markers will provide eventually a more comprehensive basis to fully map the cortex. As shown by Rajkowska and Goldman-Rakic (1995a,b) in the human brain there is variation in the extent and precise location of a cortical area. A larger sample for each species is necessary, and caution needs to apply in the interpretation of results based on small samples due to intraspecific variation and possible hemispheric assymetries in the organization of the cortex.

In the past, an accurate quantitative approach to the study of the prefrontal cortex was not available. Problems are present that involve the purely descriptive basis of outlining and evaluating cortical areas, the more restricted technical means and sampling techniques, and the small number of available brain specimens that did not allow for such effort. Computerized imaging technology, stereological sampling methods, and improved staining techniques are now available and can help to overcome many of the these obstacles. Some of these new advances have been successfully applied in other parts of the cortex, including the cingulate cortex, and the occipital and temporal lobes (Zilles et al., 1986; Armstrong et al., 1986; West and Gundersen, 1990; West et al., 1991; Schlaug et al., 1993).

Knowledge of the organization and size of individual cortical areas is necessary for the evolutionary study of the brain. The human brain is not an enlarged ape brain, nor are the ape and monkey brains enlarged or reduced versions of each other (Holloway, 1968). Different areas changed to a different extent over time under different selection pressures for each species, resulting in distinct neural organizations (Armstrong, 1990). The organization and size of individual cortical areas vary across species, and such knowledge is necessary for the evolutionary study of the brain. In particular, knowledge of the brain of the extant apes, as compared to the human brain, can assist in the identification of features that are shared among hominoids or are unique to humans and developed during hominid evolution.

In terms of absolute size, area 13 is a conservative (primitive) area in the hominoid orbitofrontal cortex. It also has similar structural features among species as was shown in this cytoarchitectonic study. It is suggested here that area 13 was part of the Plio-Pleistocene hominoid and hominid brain. A hypothetical reconstruction of this brain would place area 13 in a restricted area, occupying the most posterior parts of the medial orbital gyrus and the posterior orbital gyrus, with structural features similar to those present in the extant species.

There is variation in the size and in aspects of the organization of the frontal lobes among the hominoids (Semendeferi et al., 1997). Differences in size and aspects of the organization are present and might reflect species-specific adaptations, functional specializations, and/or major evolutionary events relating to changes in the organization of the hominoid brain. Mere consideration of relationships such as total brain size to body size is inadequate for the understanding of species-specific adaptations in behavior and underlying neural circuitry. This study revealed differences in the organization of parts of the limbic frontal cortex, involved in social cognition in species that have very similar absolute brain sizes (orangutan versus chimpanzee). It is clear that specific neural circuits or cortical areas have to be targeted and compared in closely related species.

SUMMARY

Area 13 was known to be present in the posterior orbitofrontal cortex of the macaque and to form part of the limbic system involved in behaviors related to responses to social stimuli and social cognition. It was shown here that area 13 is present in all extant hominoids and is characterized across species by the presence of conservative structural features. Anatomical differences that might relate to species-specific adaptations in behavior were found to exist in the relative size of this cortical area. The human and the bonobo were found to have a highly diversified posterior orbitofrontal cortex that includes many subdivisions of cortical areas and a relatively small area 13. In contrast the brain of the orangutan was found to have a smaller and more homogeneous orbi-

tofrontal cortex, with area 13 occupying a larger percentage of its total volume.

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